

Certificate of Analysis for NR-53944

SARS-Related Coronavirus 2, Isolate hCoV-19/Scotland/CVR837/2020

Catalog No. NR-53944

Product Description:

SARS-Related Coronavirus 2, Isolate hCoV-19/Scotland/CVR837/2020 was isolated from a throat swab from a human patient diagnosed with COVID-19, on July 17, 2020 in Scotland, United Kingdom. Deposited and labeled as SARS-CoV-2, isolate CVR-GLA-1 prior to the determination of the official name SARS-CoV-2, isolate hCoV-19/Scotland/CVR837/2020. NR-53944 lot 70041278 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with human signaling lymphocytic activation molecule (Vero-hSLAM) and incubating in Dulbecco's Minimum Essential Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) and 1% penicillin/streptomycin solution (ATCC® 30-2300™) for 5 days at 37°C with 5% CO₂.

Passage History:

VE(1)/VhSLAM(1) (University of Glasgow Centre for Virus Research/BEI Resources); VE = Vero E6 cells; VhSLAM = Vero-hSLAM cells

Lot: 70041278 Manufacturing Date: 29DEC2020

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TEST	SPECIFICATIONS	RESULTS		
Identification by Infectivity in Vero-hSLAM Cells	Cell rounding and detachment	Cell rounding and detachment		
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS- CoV-2, isolate hCoV- 19/Scotland/CVR837/2020 (GISAID: EPI_ISL_461705)	99.99% identity with SARS- CoV-2, isolate hCoV- 19/Scotland/CVR837/2020 (GISAID: EPI_ISL_461705)		
Titer by TCID₅ Assay in Vero-hSLAM Cells by Cytopathic Effect¹ (7 days at 37°C and 5% CO₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL		
Sterility (21-day incubation)				
Harpo's HTYE broth, 37°C and 26°C, aerobic ²	No growth	No growth		
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth		
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth		
Sheep blood agar, 37°C, aerobic	No growth	No growth		
Sheep blood agar, 37°C, anaerobic	No growth	No growth		
Thioglycollate broth, 37°C, anaerobic	No growth	No growth		
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth		
Mycoplasma Contamination				
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected		
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected		

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.
²Atlas, Ronald M. Handbook of Microbiological Media.
3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-53944 lot 70041278

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of two SNPs when compared to the reference sequence from EPI_ISL_461705 (see Table I below). Additionally, both the reference sequence EPI_ISL_461705 and NR-53944 lot 70041278 contained five SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-53944 lot 70041278 and reference sequence EPI_ISL_461705

Position in NR-53944_ 70041278 Sequence	Position in EPI_ISL_ 461705 Reference Sequence	Position in MN908947 Wuhan- Hu-1 Sequence	Reported MN908947 Wuhan- Hu-1 Sequence	Reported EPI_ISL_ 461705 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
14625	14625	14679	Т	Т	С	274	SNP	1	0.058824
21498	21498	21552	С	С	Т	9023	SNP	1	0.341141

Table II: Variants with different nucleotides between NR-53944 lot 70041278 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-53944_ 70041278 Sequence	Position in EPI_ISL_ 461705 Reference Sequence	Position in MN908947 Wuhan- Hu-1 Sequence	Reported MN908947 Wuhan- Hu-1 Sequence	Reported EPI_ISL_ 461705 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	187	241	С	Т	Т	N/A	SNP	1	1.000000
2983	2983	3037	С	Т	Т	N/A	SNP	1	1.000000
14354	14354	14408	С	Т	Т	N/A	SNP	1	1.000000
23349	23349	23403	Α	G	G	N/A	SNP	1	1.000000
24334	24334	24388	Α	T	T	N/A	SNP	1	1.000000

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